

The University of Chicago

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OVARY OF THE OPOSSUM FROM
BIRTH TO MATURITY AND ITS
REACTION TO SEX HORMONES

A DISSERTATION SUBMITTED TO THE FACULTY OF THE
DIVISION OF THE BIOLOGICAL SCIENCES IN CANDI-
DACY FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ZOÖLOGY
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THE NORMAL DEVELOPMENT OF THE OVARY OF THE OPOSSUM FROM BIRTH TO MATURITY AND ITS REACTIONS TO SEX HORMONES^{1, 2}

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ONE TEXT FIGURE AND THREE PLATES (TWENTY-ONE FIGURES)

INTRODUCTION

An interest in the effects of hormones on the developing ovary and accessory sexual structures was aroused by the studies of Lillie ('16, '17) and Chapin ('17), who proposed that the testis-like gonad of the female intersex freemartin was caused by a substance secreted in the male twin calf during the period in which the twins were connected by a common anastomosis of blood vessels. This substance was postulated to be a male sex hormone secreted by the testis.

Many investigators have since attempted to duplicate the freemartin condition in mammals by various methods of treatment, among which are injections of isolated hormones into the pregnant mother or directly into the amniotic cavity. The opossum has proved to be an excellent mammal for this type of investigation since the hormone application can be given directly to the young "embryo" in the pouch.

¹ The author wishes to express his appreciation to Dr. Carl R. Moore for his suggestion of the problem and his continual guidance throughout the prosecution of the research, to Dr. Sewall Wright for assistance in statistical analyses, and to Dr. Dorothy Price for helpful criticism.

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The reproductive system of the female opossum was brought to special attention by the work of Hartman ('16-'34). Hartman not only recorded the first observations on parturition and pouch entry in this marsupial, but he also described many phases of the reproductive process, including estrus cycles, ovulation, early embryo development, and some details of ovarian histology and physiology. These studies laid the foundation for more extensive investigations into the embryology of the opossum and culminated in McGrady's account in 1938, which treated, primarily, development during the gestational period; these studies contributed but little to knowledge of ovarian development, since the latter falls in the post-natal period.

The male reproductive system of the opossum has been studied by Chase ('39) who described its morphology, as well as some aspects of the interrelation between sex hormones and reproductive organs. More recently, due to their advent into the maternal pouch at such an early stage in development of the reproductive system, the pouch-young have been used by Moore ('39, 1941 a, b) and Burns ('39 b, c, d, e) for the study of the effects of hormones on sex differentiation and development.

Since the study of the effects of various hormones on ovarian development (Morgan, '41) revealed several aspects of special interest, it became necessary to make a thorough study of the normal development of the ovary from birth to maturity in order to insure a clear understanding of any possible responses to hormonal substances upon this organ. This paper contains a discussion of normal development in opossum ovaries from birth to maturity, with some consideration of responses of this organ to treatments with androgens, estrogens, and gonadotropins during various phases of its development.

THE MATERIALS

Litters for the study were obtained from adult opossums confined in outdoor pens at Whitman Laboratory on the

University of Chicago campus and from mothers shipped from Michigan, Arkansas, Missouri, Kentucky, and Florida. Litters from Whitman Laboratory were usually observed within 24 hours after birth, and the mothers removed to an indoor pen. At regular intervals some of the young were measured, weighed, killed and preserved, for the purpose of establishing a normal key for pouch-young of unknown delivery date (Moore and Bodian, '40). Sixty-four female opossums, of which the reproductive tract was serially sectioned, were employed in the study of ages 1 day to 100 days. Of these animals twenty-six were untreated normal, twelve were treated with estrogen, twenty-one with androgen, and five with gonadotropin. Sample data for twenty of these animals are recorded in table 1.

Thirty-nine serially sectioned ovaries of juvenile and adult ages were studied; of these, twenty were untreated controls, four were treated with estrogen, five with androgen, and ten with gonadotropin. Data from twenty-one of these ovaries are recorded in tables 1 and 2.

The tissues were fixed in either Bouin's fluid or Formalin-Zenker, then serially sectioned, mounted and stained with either Mallory's connective tissue stain (Azan), the short Foot method for silver impregnation of reticulum counterstained with azo-carmin, Ehrlich's hematoxylin, or Harris' hematoxylin. The study of the reproductive tract and its response to hormones of some of these animals has been published by Moore ('41 a).

Androgens. The androgens, testosterone and t-propionate, were administered to the early pouch-young by inunction with a lanolin base ointment so that absorption of the hormone was percutaneous, and to later ages by subcutaneous injections. Necessarily, all the pouch-young in one litter had to receive the same treatment. The sex of the young animals was unknown at the beginning of the earlier treatments, but by day 12 it was possible to distinguish the sex by the slight scrotal elevation anterior to the phallus on the male and by a definite

TABLE 1

Sample data for young female opossums—normal and treated.

SER. NO.	AGE AT DEATH	WEIGHT	SNOUT-RUMP LENGTH	HEAD LENGTH	TREATMENT	OVARIAN SIZE	WT. REPRO-DUCTIVE TRACT
Normals							
	(days)	(gm.)	(mm.)	(mm.)	(days)		(gm.)
1 A	5	0.39	16.3	6.3		6,551 ¹	
2 A	10	0.82	21.9	8.9		9,348 ¹	
6 A	17	1.17	28.7	11.4		19,076 ¹	
18 (6)	31	3.85	44.2	16.4		37,098 ¹	
87 B	44	11.35	70.0	24.5		391,952 ¹	
83 B	67	24.30	96.0	32.5		662,796 ¹	
79 B	75	37.00	101.0	36.0		537,200 ¹	
82 B	100	145.00	203.0	60.3		1,006,098 ¹	
228	125	320.00				0.0304 ²	0.4700
229	125	300.00				0.0248 ²	0.3600
178	150	410.00				0.0602 ²	0.3380
179	150	390.00				0.0760 ²	0.4166
Androgen-treated							
96 B	16	1.65	36.5	11.5	3-15 TO	22,318 ¹	
101 B	30	4.06	50.0	16.3	3-15 TO; 16-29 TP	78,852 ¹	
107	45	12.11	72.2	24.6	3-15 TO; 16-44 TP	198,150 ¹	
66	62	16.50	89.5	29.3	10-31 TP	366,714 ¹	
117	95	216.00	221.0	65.2	3-15 TO; 16-93 TP	811,552 ¹	
174	150	400.00			2 mg. TP — 13 days	0.0430 ²	5.9300
175	150	350.00			2 mg. TP — 13 days	0.0400 ²	4.7200
Estrogen-treated							
49 B	17	1.50	33.5	11.0	5-16 EsO	19,022 ¹	
108	30	3.81	48.6	15.8	6-29 EsO	56,544 ¹	
111	46	10.94	73.4	24.1	6-45 EsO	328,476 ¹	
170	150	290.00			100 IU PB — 12 days	0.0622 ²	3.1600
171	150	320.00			100 IU PB — 12 days	0.0380 ²	2.5500
Gonadotropin-treated							
99 B	17	2.00	37.0	11.6	10-16 Gd	29,244 ¹	
113	30	2.93	43.5	15.2	15-29 Gd	91,172 ¹	
116	38	6.40	59.7	20.1	15-37 Gd	170,398 ¹	
90 (41)	100	130.00	173.0	52.9	85-99 Gd	804,307 ¹	

¹ Planimetric volume units.² Weight 2 ovaries in gm.

TO, testosterone ointment; TP, t-propionate injection; EsO, estrogenic ointment; PB, Progynon B injection; Gd, gonadotropic injection.

mammary gland pattern on the female. The first treatments were started by day 3 before sexual differentiation.

Estrogens. The principal method of applying the estrogens to the early pouch-young was cutaneous application of estradiol in ointment, applications starting by day 5; although subcutaneous injections of Progynon B in oil solution, were used in some cases of pouch-young, they were used exclusively in the juvenile and adult stages. The estrogens were found to be very toxic to the pouch-young and many fatalities resulted from high dosages used in earlier experiments.

Gonadotropins. The material at hand on early development (day 1 to 100) is rather limited, but that of juvenile and adult anestrus stages is more extensive. Only a few of the experiments will be discussed in this paper since the effect of gonadotropins on the ovary is to be the subject of a future communication. Gonadin, containing the gonadotropic principles of pregnant mare serum, was administered to the opossum during all stages. This preparation has been shown to approach the effectiveness of the gonadotropic substances of the pituitary and contains both the follicle stimulation and luteinizing principles.

THE NORMAL DEVELOPMENT OF THE OVARY

Gonads at birth. The gonads were extremely small and each was situated in the posterior one-fourth of its respective elongated genital ridge located on the medial surface of the functional mesonephros; the latter organ persisted as the excretory organ of the newborn until approximately the twelfth to fourteenth day. The genital ridge began about one-eighth of the distance from the anterior end of the mesonephros and extended two-thirds of its length. The anterior three-fourths of the genital ridge was rete and the remaining posterior one-fourth was occupied by the gonad. The third division, mesenteric ridge, mentioned by Allen ('04), was scarcely detectible at this age.

The mesonephros measured about 1.5 mm. in length from the anterior to the posterior end and was covered by a distinct

peritoneal membrane. The membrane was clearly separated from underlying mesonephric tubules and corpuscles but not so clearly defined from underlying loosely arranged stromal cells.

In the 1-day opossum, primary sex cords, tunica albuginea, germinal epithelium and rete were all present. These will be discussed in order.

The medullary (primary) sex cords had already formed and were growing centripetally in dovetail position at the center of the gonad as may be seen in figure 1. Most cords were separated from the germinal epithelium by the primary tunica albuginea, but a few of the medullary cords were still attached to the peritoneum and this was the only direct evidence from the post-natal ages that the medullary cords had their origin from the germinal epithelium. The process of medullary cord formation consisted of invagination of the germinal epithelium, shown by the tendency to a line of weakness or rudimentary lumen in the center of the early developing cord near its basal connection. Growth of the cord seemed to be caused by mitoses of the cells in the cord itself and by development of the cords at their points of attachment to the peritoneum.

The spherical or oval shaped nuclei of the epithelial cells of the medullary cords were generally larger than those of the cells of either the germinal epithelium or the primary tunica albuginea, and were embedded in a non-fibrous, homogeneous cytoplasm without visible cell boundaries (fig. 1). Occasionally larger cells which were fully differentiated into distinctive primitive germ cells appeared in the medullary cords. Each had a spherical nucleus, usually containing prominent nucleoli and fine strands of chromatin material which were in contrast to the irregular granular chromatin masses of other cells. The germ cells did not have clearly defined boundaries at this age. There was no indication of granules characteristic of secreting cells.

The peripheral primary tunica albuginea was forming by day 1 between most of the medullary cords and the germinal

epithelium. It appeared to arise from proliferations of mesenchyme cells from the germinal epithelium to which some of the cells were still attached. The fine fibril-like cytoplasm was distinctly different from the cytoplasm of the epithelial cells in the medullary cords. The germinal epithelium in some regions appeared as a very thin peritoneal wall containing elongated flat nuclei that paralleled the gonad. In more active regions, near the attachments of the gonad to the mesonephros, the cells were columnar and cuboidal (cf. fig. 1).

Rete cords grew in a tortuous course from the peritoneum in the rete portion of the genital ridge. Their manner of origin and growth was similar to that of the already described primary cords, that is, by invaginations of the peritoneum; however, these rete cords were not as abundant in any given area as were the primary cords and did not appear to be arranged segmentally. Some of them extended across the rete ridge and approached the epithelial wall of Bowman's capsule, while others were already oriented posteriorly toward the gonad but had not yet reached it. The epithelial cells of the rete cords in these early ages appeared similar to the epithelial cells of the primary cords, and this was to be expected since both had similar origins occurring at approximately the same time. The invagination of the peritoneum left the center of the cord with a line of weakness in which there were no nuclei. The darkly staining nuclei of the rete cords were arranged near the periphery of the cord, and their apices were placed toward the future lumen. The smaller, lightly staining mesenchyme cells of the rete ridge were easily distinguished from the cells of the rete cords. Primordial germ cells were not detectible in the rete ridge.

The 5-day ovary. The gonad at this age was clearly distinguished as male or female. The testicle revealed heavy, well circumscribed, elongated primary sex cords and the germinal epithelium was for the most part flattened and inactive and did not contain primordial germ cells. The ovary was composed of less discrete and less regular but more numerous primary sex cords and its germinal epithelium was composed

of several layers of cells in which numerous primordial ova were present. The tunica albuginea, indicated in both, was less compact in the ovary.

Secondary sex cords had made their appearance in the 5-day ovary. Formed by invaginations of the peritoneum, they appeared as cords of cells retaining no lumina from the invagination, but only a rudimentary line of weakness near the basal portion (cf. fig. 7). In the opossum these ingrowing secondary cords were not continuous with the primary cords but were separated from them by the primary tunica albuginea.

The oögonia had a higher frequency in the secondary sex cords, the primary sex cords, and the germinal epithelium than did the primordial germ cells in the 1-day opossum, but the epithelial cells were still superior in number. This indicates that epithelial cells of the primary cords can differentiate into germ cells (cf. fig. 7). Thus oögonia came directly from the germinal epithelium or indirectly via the cords. The oögonia were similar to the primordial germ cells described in the 1-day gonad, but differed in that the lightly staining cytoplasm had a delicate limiting boundary, and the nuclei were somewhat larger. Stromal cells were somewhat more abundant throughout the ovary than at day 1.

The mesonephros had increased to about 1.75 mm. in length and had a greater dorsal to ventral measurement than at day 1. The relationship of the 5-day ovary to the mesonephros is illustrated in figure 2. The ostium of the müllerian duct was situated in the most anterior part of the mesonephros. Rete cords had extended to the mesovarium of the ovary but were no more abundant than in the preceding stage.

The appearance of secondary or cortical cords in the ovary by the fifth day suggested the careful examination of the testis for similar formations. Embryonic testes of all groups below mammals have shown secondary cord proliferation as a transient feature in their development, and Gruenwald ('42) has reviewed this problem for mammals. The results of the examination follow:

The 5-day-old opossum testis was bounded for the most part by a thin, flat, serous membrane; however, in several places, a more conspicuous columnar germinal epithelium was seen, frequently near the mesenterial attachment. Occasionally down-growths resembled the typical secondary ovarian proliferations and produced distinct epithelial aggregates just below the surface level. Smaller numbers of such formations were seen in the 7- and 12-day testicles. The similarity in time of appearance and in form suggested that these represent transient secondary cord proliferations on the part of the developing opossum testis. The problem deserves more attention and an attempt will be made to study it in a more extensive series of closely graded testes.

The 7-day ovary. Secondary sex cords were increased in number, though some were still in the elementary process of germinal epithelial invagination and all cords continued to be separated from the primary cords by the primary tunica albuginea. The primary sex cords seemed to be grouped together near the mesovarium.

The rete cords showed a marked increase in development over the earlier stages. They could be traced over a devious course running from the peritoneal wall of the rete ridge either to Bowman's capsules, or to evaginations from the capsules, (fig. 9), branching to other capsules, collecting into one rete duct (Morgan, '41), and continuing posteriorly toward the gonad through the mesovarium into the medullary region of the ovary. The evagination from Bowman's capsule, shown in figure 9, did not contact the rete invagination from the peritoneum also shown in figure 9. Some of the rete cords had rudimentary lumina from the peritoneum and even a connection to the cavity of the Malpighian body. The lumen was not perceptibly continuous to the gonad. The anterior tip of the mesonephros had begun to degenerate with the disruption of the mesonephric tubules and the appearance of more stromal cells.

The 10-day ovary. Further degeneration of the anterior mesonephric tubules and a greatly increased amount of stromal tissue over that of the preceding stage was evident.

The rete cords were more distinct than before, with lumina perceptible nearly all the way into the ovary. The nuclei were somewhat elongated and irregular in shape with longitudinal axes facing the lumen of the tubule. Formation of the lumen appeared to be caused by the cells pulling away from the center line of weakness. The primary sex cords were represented now only by groups or clumps of cells which, when traced serially, proved to be actually solid cords of cells.

The cortical cords (secondary cords) had increased in size. Unusual cord formations, which we suggest to be secondary cords with open lumina, were sometimes present near the hilus in some ovaries. The cords possessed open lumina from the germinal epithelium and extended toward and sometimes into the medulla of the ovary. Varying amounts of the distal ends, made of solid cords, never appeared connected with the rete. Their location in the ovary proper, their cell structure — differing from that of the rete, and lack of connection to the rete led to their designation as secondary cords. Possibly their formation was caused by an overactive invagination of the germinal epithelium and their location, near the hilus, resulted from the activity of the germinal epithelium which is frequently greater at this location than elsewhere. These have probably been confused with rete cords by some investigators.

The first appearance of the definitive tunica albuginea in the ovary of the opossum was made at this age when some of the earlier formed secondary cords had become separated from the germinal epithelium by connective tissue. (The definitive tunica albuginea is formed between the germinal epithelium and the secondary sex cords and is a distinct ovarian structure without a homologue in the testis. The primary tunica albuginea is present between the secondary cords and the primary cords in the ovary and is homologous to the tunica albuginea in the testis.) It appeared that many of the mesenchyme cells of the definitive albuginea had been proliferated from the germinal epithelium whose predominating cell was elongated and perpendicular to the gonad with a fibrous strand of cytoplasm extending into the tunica albuginea. So many

of these cells were leaving the germinal epithelium that it gave the appearance of being several layers thick.

There was an increase in number of oögonia in the germinal epithelium and this advance in development seemed to have taken place in both the primary and secondary cords at about the same time, in spite of the fact that the primary cords developed first. Some of the oögonia in the secondary and primary cords had developed into the oöcyte stage with chromatin threads in the synapsis form (Winiwarter, '00).

The 12-day ovary. The germinal epithelium, less active than previously described, was only 1-cell layer in thickness and was made up of cells having spherical or cuboidal nuclei. It was no longer possible to detect so many of the elongated nuclei moving into the definitive tunica albuginea, but oögonia were being proliferated, and the number becoming oöcytes had increased only slightly over that in the earlier age described above. Only a few of the secondary cords remained attached to the germinal epithelium; the primary tunica albuginea had thickened, and the rete cords were much the same as those of the 10-day period, but the tubules were patent in the intra-ovarian portion.

The 16- to 17-day ovary. Many of the mesonephric tubules and corpuscles had degenerated and in the anterior mesonephros only traces of these remained, so that most of the rete tubules were connected directly to the remaining mesonephric tubules or epoöphoron. Hence, in the opossum female of this age a lumen could be traced from the intra-ovarian rete, through the epoöphoron, and into the Wolffian duct. Thus the opossum female has a duplication of the functional connections of the male. The intra-ovarian rete for the first time showed a distinct lumen which measured 4-5 μ in diameter and was somewhat larger in the extra-ovarian portion of the rete—the measurement being 8 μ . A trace existed of the former connection of the extra-ovarian rete to the peritoneal wall and a lumen was discernible in opossum 6 A, but this was not true in opossum 33 B.

Primary cords of solid cells were discernible in some of the ovaries, but from this age on, these cords seemed either to atrophy completely in some ovaries or to be maintained in variable amounts in others. The rete had grown into the ovary so that it was either in close proximity to the primary cords or in direct contact with them, though a common connection was not seen at this age. The connection of the rete to a remaining Bowman's capsule in a 17-day gonadin-treated female is illustrated in figure 10; in the untreated animals the connections are similar. This short segment differed from the usual rete and gave rise to the opinion that this component was derived from the outpouching of the epithelium of the mesonephric corpuscle. Figure 9, from an untreated animal, age 7 days, shows one of the evaginations of the capsule epithelium approaching a rete invagination from the peritoneal wall. As seen in serial section, the two did not come into contact in this instance.

The germinal epithelium was similar to that of the 12-day stage; however, it was no longer connected to most of the secondary cords. There had been an increase in oögonia by mitoses and proliferations from the germinal epithelium, both in the strömal tissue of the ovary and in the sex cords. For the first time some of these were surrounded by a single row of mesenchyme cells to produce primordial follicles.

The 24-day ovary. The entire mesonephros was rapidly degenerating with a disappearance of mesonephric corpuscles, and only vestigial portions of the paroöphoron remained. Some of the epoöphoron ducts were larger and their connections with the extra-ovarian rete tubules were very near the mesovarium, due possibly to growth of the evaginated portion from Bowman's capsules toward the ovary and to extension of the growing ovary to enclose more of the extra-ovarian rete. The cells of the epoöphoron were easily distinguished from the cells of the rete, since the nuclei of the epoöphoron stained much lighter, were more spherical and less crowded than the nuclei of the rete. A portion of the intra-ovarian rete is shown in figure 15; it is in direct contact with primary cords. Con-

nection of the rete cords with the peritoneal wall was not apparent in female opossum 10 A.

Proliferations of oögonia from the germinal epithelium were more rapid than in any preceding age; this germinal epithelium was now at least 2 cells in thickness and mitoses were much more prominent than earlier. The epithelial cells predominated in number over oögonia cells at the time of formation of the secondary cords. At 24 days the condition was reversing. Germ cells were dividing outside the germinal epithelium, as shown by some mitotic figures and by many oögonia occurring in groups of two (cf. fig. 15). This description of the secondary cords does not apply to the few newly forming secondary cords in which the epithelial cells were smaller, less numerous, and the nuclei stained more intensively — similar to secondary cords at day 5. Long strands of fibers from connective tissue cells, coming from the mesovarium, were invading the ovary throughout the medullary area and between some of the secondary cords. Primary cords were still solid and portions are shown in figure 15.

The 31-day ovary. The germinal epithelium was again inactive, which indicated a rhythmical character. In this case the activity was less than at day 24, but similar to days 16–17. The secondary sex cords showed a further diminution of epithelial cells, as compared with earlier stages, and a greater increase of oögonia and oöcytes was apparent (fig. 4 of day 31 and compare with fig. 15 of day 24). The epoöphoron ducts made their connection with the extra-ovarian part of the rete tubules in the mesovarium, and both tubules retained lumina. Secondary sex cords, with short open lumina, extended from the periphery of the ovary inward toward the center, but did not connect with the rete; however, primary cords were connected to short branches from the rete.

The 44-day ovary. The previous epithelial-celled secondary cords were composed only of enlarged oögonia, in rather discrete masses of different sizes surrounded by connective tissue (cf. figs. 3, 5, and 17). These large masses of polyhedral-shaped (fig. 12) cells could be designated “cell nests”;

the nests appeared to be formed by the action of stromal cells growing into and breaking up the secondary cords. Just outside these cell nests single germ cells were surrounded by single rows of follicular cells which appeared similar to connective tissue cells (cf. center fig. 17). The primary cords did not contain the cell nests, for the oögonia, although present, remained single and scattered. Usually all cells of each nest had delicate boundaries and were in the same prophase stage — either deutobroch, leptotene, pachytene, diplotene or the resting diffuse stage. Meiosis beyond the prophase or the diffuse resting stage was never seen (cf. figs. 5 and 12). Degenerating and pycnotic germ cells were numerous in the cell nests and were usually remote from the periphery where the vascular supply was limited.

An active germinal epithelium, made up of columnar and cuboidal cells, contained many oögonia undergoing growth and pushing out into the definitive tunica albuginea. Some of the oögonia had moved as far as the mesenchyme cell region between the nests. The cells of the germinal epithelium were differentiating into germ cells in a manner similar to that in the secondary cords. Many germ cells were noticeable although only a few mototic figures were present, and thus germ cells seemed to differentiate suddenly from the undividing epithelial cells and then to increase in size. Cells of both the secondary sex cords and the germinal epithelium differentiated, grew, and became germ cells only after cessation of mitosis. The intra-ovarian rete had branched to join many primary cords within which could be seen developing ova. The primary cords of opossum 87 B were short and were more atrophic than in some of the ovaries of other opossums, and the rete had lost connection with the coelom, but secondary cords with open lumina were again located near the hilus. These did not join the rete.

The ovary had increased in volume, as indicated by planimetric measurements, to $60 \times$ the volume of the 5-day ovary and to $10 \times$ the ovarian volume at day 31. The most rapid rate of increase in volume of all ovarian development occurred

during the 13 days preceding day 44 and paralleled the increased size of the many oöcytes of the cell nests (cf. text fig. 1).

The 67-day ovary. The cell nests were surrounded by a more compact sheath of mesenchyme than at day 44 (fig. 3). The oöcytes composing the nests had increased in both size and number, and many were degenerating. Also, oöcytes outside the nests were sometimes degenerating. The active follicular formation which was apparent here and at day 100 will be described in the 100-day ovary. The epoöphoron ducts

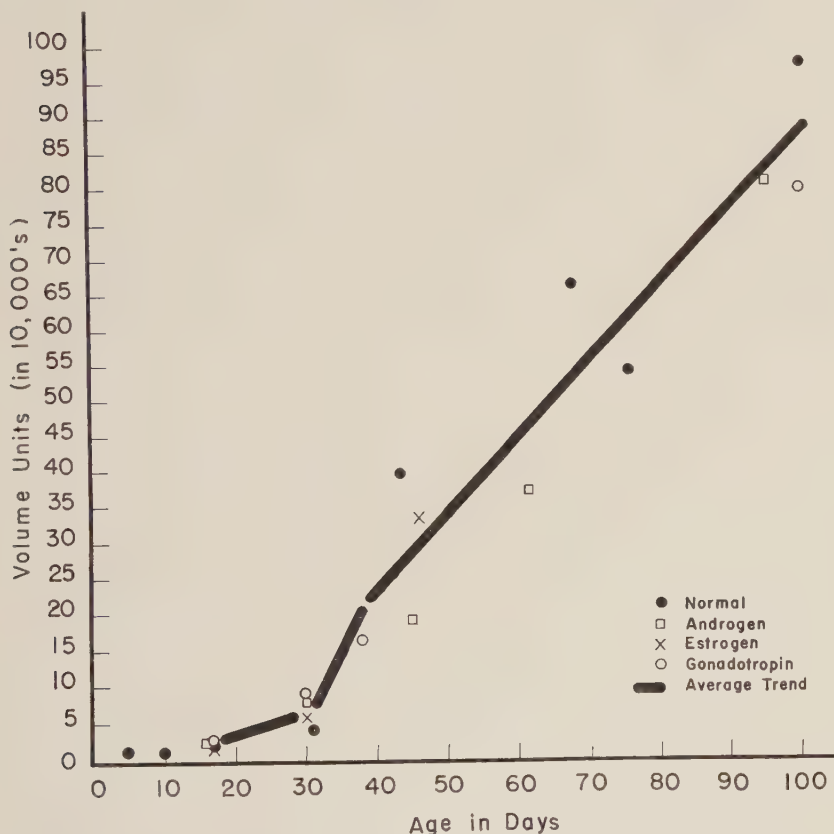


Fig. 1 The relation of "embryonic" ovarian volumes, as determined by planimeter, to age in days of normal animals and of those treated with androgens, estrogens and gonadotropins.

ended blindly in the anterior part of a very much degenerated stroma, but they could be traced to a connection with the rete in the mesovarium. The rete appeared free of germ cells. The innermost ends of the branches of the intra-ovarian part of the rete showed connections to primary tubules and frequently large oöcytes were present within the primary tubules.

The 100-day ovary. The volume of the 100-day ovary, as shown by planimetric measurements, had increased $150 \times$ the volume of the ovary at day 5, $20 \times$ the volume at day 31 and $2.5 \times$ that at day 44.

A moderately active germinal epithelium surrounded the 100-day ovary. Granulosa cells of the larger follicles were now arranged in a layer 2 to 3 cells in thickness (cf. figs. 5 and 20). Active follicular formation from the cortical cell nests was in progress at 67–100 days, but primordial follicles were observed as early as days 16–17. The manner of follicular formation was as follows: Reticular fibers (fig. 14), followed by stromal cells (fig. 5), grew into the oöcyte cell nests and segregated individual oöcytes from the nests. This was shown by the short Foot method of silver impregnation of reticulum, counterstained with azo-carmin. Stromal cells, which appeared originally from the germinal epithelium and from the cells moving in from the mesovarium as described at day 24, became squamous follicular cells surrounding the oöcyte; these then differentiated into a layer of columnar granulosa cells surrounding the ovum, and finally underwent mitosis to form a stratified epithelium of granulosa cells. The change from a mesenchymal cell to a granulosum cell, under a possible inductive influence from the germ cells, involved an enlargement of the nucleus by an increase of nuclear fluid and a dispersion of the chromatin material. The nucleoli did not appear to change. A complete cell nest was divided and partitioned into two or more small nests by “spear-heads” sent in by the invading stroma. The stromal cells seemed thus to play an active role in the ovary. The above suggests a method of primary follicle formation which appeared to be common for both the oöcytes found in the cortical cords and those oöcytes found in the

stroma and derived directly from the germinal epithelium. A review of the literature did not reveal a method similar to the one described.

The cell nests contained many large vacuoles left by shrunken, degenerating cells still seen at the periphery of these vacuoles (fig. 5). Cell counts demonstrated more diffuse or resting stage oöcytes in the cell nests at this age than in the cell nests at day 67 when earlier prophase stages dominated. The inchoative theca interna was distinguished only by the connective tissue cells becoming loosely arranged around the follicles (cf. fig. 5). The ovum in the larger follicles was enclosed by a thin membrane, the zona pellucida (fig. 5).

Primary tubules were connected to rete and contained large developing oöcytes. Occasionally some of these oöcytes became so large that the undifferentiated cells of the primary cords were stretched out around them causing the tubule to appear much the same as primary follicles surrounded by one or two layers of granulosa-like cells (figs. 13 and 20). In serial section, the primary cord, at the lower center of figure 13, was seen joining the rete. After the oöcytes grew to the size shown in the primary tubule in the lower center of figure 13, they began to degenerate as shown by the oöcyte in the tubule in the upper center of figure 13. In serial section, a large percentage of the follicles found in the medullary portion of the ovary were seen to be enlargements of the primary tubules. Primary tubule cells differed from the rete tubule cells in that the nuclei of the former stained less intensely, were more spherical, and were arranged irregularly in two or more layers; whereas the rete cord cells were elongated and in one layer (cf. figs. 8 and 13). The large, regular and continuous lumen of the rete stopped abruptly at the connection with the primary cord, which had only an irregular and sometimes rudimentary lumen (cf. fig. 8).

The 125-day ovary. The ovary for the first time had follicles containing initial formations of antra. This stage of development, secured subsequent to the preliminary report of these studies (Morgan, '41), indicated that antrum formation can

be placed earlier than 5 months. This observation is significant since it is one of the first histological signs indicating ovarian, hormonal function. This makes a natural division point and we have arbitrarily started the juvenile stage at day 125.

Micrometer measurements revealed that granulosa cells of antrum-bearing follicles were larger than granulosa cells of follicles prior to beginning antrum formation. The structure of cells surrounding the follicles was now like that of cells of typical theca interna. A very few fine fibrils, continuous with the theca externa and ovarian stroma, were in evidence between the theca interna cells. The zona pellucida was thicker and more prominent; blood capillaries were increased in number throughout the stroma and theca interna; many of the larger follicles were undergoing atresia; small cell nests were infrequent, and connections still existed between the rete and the primary tubules, within which were many of the follicles of the medullary region. Germinal epithelium, composed of a single row of cells, was active near the hilus but only moderately so elsewhere.

The 150-day ovary. The ovary had doubled in weight since the 125-day age (table 1). Histological examination showed that most of this increase was due to the growth of some follicles into larger follicles, of which only a few had antra and/or intercellular accumulations of follicular fluid among the granulosa cells. The germinal epithelium consisted of a single layer of flattened cells and was comparatively inert, excepting for the continued activity near the hilus. Cell nests were no longer visible. In figure 18 it can be seen that primary tubules are connected with rete tubules and large ova are present in the primary tubules.

The 240-day ovary. The process of ovarian development up to the one hundred and fiftieth day involved, among other elements, the formation of the secondary cords, the growth of the secondary cord cells into definite oöcyte nests, the gradual dissolution of these nests of cells into primary follicles by invasion of stromal cells and reticular fibers, and the eventual disappearance of the nests by day 150. Antrum bearing fol-

licles appeared by day 125 and the opossum could then be said to be in the juvenile stage. The 240-day opossum was autopsied in December during the season of anestrus. As a result, less ovarian activity than at day 125 was evident and antra were not visible in the follicles. This ovary, therefore, as can be seen in figure 11, was covered by an inactive germinal epithelium; many inactive young follicles were present and atretic changes were frequently evident. Rete tubules with open lumina were still connected to primary tubules.

The anestrus adult ovary. The anestrus ovary was only one-eighth to one-fourth the weight of the ovary during the breeding season and had a rather definite cortex and medulla, open lumen rete cords—located in the medullary area, and primary or medullary tubules (derived from the primary sex cords). It revealed a lack of corpora lutea.

In the cortical area, the germinal epithelium was most active near the hilus where the cells were perpendicular to the body of the ovary and a few germ cells, but more connective tissue-like cells, were seen proliferating into the tunica albuginea. In general, elsewhere, the germinal epithelium was less active and, in some places, was composed of flattened cells parallel to the surface. Primary follicles, follicles with large antra, follicles undergoing atresia, polyovular follicles (as described by Hartman, '26 b), and pycnotic ova were all seen in the cortex.

The primary tubules were not confined exclusively to the medullary region of the ovary; they were more frequently observed in the medulla near the rete, but were also found twisting throughout the cortical region between the follicles; the latter location perhaps accounts for the erroneous classification of these primary cords as anovular follicles formed from secondary cords (League and Hartman, '25). Structurally these tubules were similar to the homologous seminiferous tubules in the male, in that in some opossum females they were connected to the rete, had a distinct basement membrane, and basal cells which grew, moving toward the center of the tubules. They differ in the respect that some of the cells of

the tubules were ova which developed to a moderately large size, but never to the size of ova of regular follicles; these ova then degenerated and became pycnotic (cf. fig. 16) due, perhaps, to the lack of nourishment. Closely graded studies of serial sections from embryo to adult revealed further that these tubules were from the primary set of sex cords. The primary tubules were not always connected, causing some to seem short and to appear similar to follicles. These fragmentary primary tubules may be caused by action of stromal cells penetrating the primary cords similar to that previously described in the dissolution of cortical cords; they have led to confused interpretations. Typical antrum formation in the primary tubules was not observed, and the cells of the tubules in the adult ovaries did not surround the ovum in a cumulous oöphorus; these observations suggest that cells in the primary tubules may not develop as granulosa cells. Primary tubules were observed in variable amounts in six out of eight serial sections of untreated adult ovaries, for as mentioned before they do not appear in all ovaries after the sixteenth day. Figure 21 is a photomicrograph at very low magnification of a typical anestrus ovary, and primary tubules can be seen, but are more apparent in figure 16 under higher magnification. Typical theca interna cells surround these primary tubules.

The rete tubules, generally located in the medulla near the hilus, were formed in a cylindrical mass of anastomosing hollow tubules lined with a single layer of epithelial cells having elongated nuclei. The tubules were separated by densely arranged connective tissue fibers (cf. figs. 6 and 19). The definitive tunica albuginea enclosed the ovary between the germinal epithelium and the cortex. The connective tissue fibers were coarse and were densely arranged parallel to the surface of the ovary.

It was mentioned above that the anestrus ovary appeared somewhat active as indicated by histological findings. A further direct check on the anestrus ovarian activity was made by ovariectomy and uterine weight determination. An adult anestrus opossum was anesthetized with sodium amytal and

by aseptic surgery was bilaterally ovariectomized and unilaterally hysterectomized. The removed control right uterus was weighed and measured, and the comparison in situ with the left uterus revealed no representable difference. Thirty days later, at autopsy, the left uterus was carefully weighed, measured, and found to be 33.1% less in weight (0.590 gm. for the control right uterus against 0.394 gm. for the left uterus) and less in gross measurement than the right uterus. Thus the anestrus ovary was apparently secreting some estrogenic substance during the anestrus season that exercised an influence on the uterus.

The ovary of the breeding season. An extensive study of the ovary was not made for all periods of the breeding season. However, the typical ovary of this season weighed four to eight times the anestrus ovary. Studies from serial sections showed, in contrast to the condition of the anestrus ovary, the presence of corpora lutea, and much larger follicles and follicular antra. The germinal epithelium was not decidedly different. The corpora lutea, formed during the breeding season after ovulation, were made up of both luteinized granulosa cells and theca interna cells. The thecal cells appeared to be derived from the ovarian stromal cells as the granulosa cells were suggested to be.

EFFECTS OF ANDROGENS ON THE OVARY

The study of possible influences of hormones upon the embryonic development of the ovary and upon the juvenile and adult organs, involved treatments with androgens, estrogens and gonadotropic substances. In addition to histological study, a comparison was made of the weights of ovaries when the ovaries could be dissected, and in younger specimens, planimetric determinations of volumes were made from serial sections. These treatments, together with the study of normal development from birth to maturity can be divided into four distinct periods, much the same as was done with the opossum testis (Moore and Morgan, '42), and it appears advantageous to discuss the treatments and results in relation to such

periods as: 1. The undifferentiated gonad through sexual differentiation — from day 1 to day 100. 2. The juvenile stage — from the first appearance of antra to the mature ovary, i.e., from about day 125 to about 1 year of age. 3. The adult anestrus stage — the non-breeding season extending from about June to February in the Chicago area. 4. The breeding stage — from about February to June. The latter stage was not utilized in hormonal treatments.

Androgenic treatments up to 100 days. Studies of the effects of androgens on the embryonic development of the ovaries of twenty-one treated females were compared with studies of adequate normal controls. Some treatments began on day 3 and continued to day 100 when the ovary was well differentiated, and others for shorter periods fell within these ages; a few of these animals are listed in table 1. Autopsies began on day 16 and continued at intervals to about day 100. The androgens were administered daily to the pouch-young in an ointment containing testosterone or t-propionate (2 mg. per gram), or by subcutaneous injections of t-propionate in sesame oil. The cutaneous ointment application was usually employed up to about day 16, after which, subcutaneous injections could be satisfactorily made. The dosages by ointment application were variable and difficult to approximate accurately, since the dosage increased as the body surface increased. The subcutaneous injections between day 16 and day 70 were in daily doses of 0.25 mg. of t-propionate in 0.05 cc. of sesame oil; after day 70 the total daily dosage was 0.5 mg. in some cases and 5.0 mg. in others.

The volumes of the ovary, as determined by planimetric analysis and discussed later, were not significantly different from the controls (cf. text fig. 1 and table 3).

Cortical areas of the ovary did not appear to show any increase or decrease in size over those of the normal control. In the secondary cords of one of the 24-day androgen-treated ovaries there was a greater proportion of differentiated oögonia to undifferentiated epithelial cells than in the untreated ovaries, but since this was not a consistent finding, it

does not appear to be significant. Comparisons of germinal epithelial activity with that of the untreated controls indicated neither an increase nor an inhibition of activity by the androgens.

The medullary tubule diameters, measured at all stages in those ovaries in which they persisted, were not increased over those of the controls by the treatment with androgens, with the exception of the 95-day treated ovaries. There was a slight dilation of some of the tubules (0.052 mm. for the untreated 100-day against 0.070 mm. in one 95-day treated and 0.064 mm. in another 95-day treated) characterized by an increased amount of cytoplasm in the cells and by an accumulation of debris from degenerating cells (figs. 8 and 13). It was not possible to determine whether the tubules were lengthened. Medullary tubules were variable in size and number in the ovary but this slight dilation did not appear to be significant. The rete exhibited only slight stimulation, but androgenic stimulation was noted in the epoöphoron ducts connected to the rete up to day 44; after this age the effect was not noticeable. An average of the ocular micrometric measurements of ten of the larger germ cells at all stages from 16 to 95 days was computed and was found to be similar in both treated and the untreated controls. Apparently androgens neither speeded up nor inhibited the development of the oögonia.

The mesonephros from days 30–45 was increased in size due to hyperplasia of the connective tissue. This increase, however, did not compare with the increase seen in the estrogenic treatments to be discussed later. A “renotropic” effect by the androgens on the mesonephric tubules was manifested by dilation of the tubules and by increased height of the cells composing the epithelial lining.

Androgenic effect on juveniles. Androgenic treatments of two 150-day animals showed an average ovarian weight decrease of 37% as compared to that of two untreated controls (table 1). This percentage is based on average ovarian weights compared to average body weights. Histological studies showed, in contrast to the untreated controls, a total

lack of follicular antra, and an increased atresia of follicles and ova. The medullary tubules were not stimulated since average measurements in the treated were 0.076 mm. and in the control, 0.080 mm. The average weight of the treated reproductive tracts was $14 \times$ that of the untreated control, showing that at this age the uteri and lateral vaginal canals responded markedly to the androgenic treatments.

Androgenic effects on anestrous ovaries. A test was made to determine the influence of androgens on the adult ovary during the anestrous period upon three females from which the right ovaries had been removed under sodium amytal

TABLE 2
Hormonal treatments of anestrous adult opossums.

SER. NO.	WT. RIGHT OVARY AT OPERATION	TREATMENT (DAILY)	WT. LEFT OVARY AT AUTOPSY	COMPARISON LEFT TO RT. OVARY (RT. OV. = 1.00)	WT. REPRO- DUCTIVE TRACT
	(gm.)		(gm.)		(gm.)
2 (10) A	0.0710		0.1200	1.549	
3 (11) A	0.0548		0.0946	1.726	
121 A	0.0438	1 mg. TP — 10 days	0.0826	1.886	6.02
123 A	0.0840	1 mg. TP — 20 days	0.0664	0.799	5.00
125 A	0.0688	5 mg. TP — 20 days			16.67
A 5	0.0890	50 IU PB — 12 days	0.0620	0.697	22.30
A 6	0.0438	100 IU PB — 12 days	0.0378	0.863	15.46

TP, t-propionate injection; PB, Progynon B injection.

anesthesia prior to treatment; following daily treatments with t-propionate, the remaining left ovary was compared with its previously removed control partner. At least 10 days were allowed for the animals to recover from the operations before treatments were started, so that autopsies were made approximately 30 days following the initial operations. Further controls were provided by ovaries from two females subjected to similar operations but not treated with androgens. It can be seen in table 2 that unilateral ovariectomy led to apparent compensatory hypertrophy of the remaining ovary during the period of 30 days, as manifested by an average

gross ovarian weight increase of 63.7% and a 150.0% increase in size of some follicular antra.

The ovary of opossum 121 A, treated for only 10 days with t-propionate (table 2), showed an 88.6% increase over the control ovarian weight, but this does not appear to be significantly more than the weight resulting from possible compensatory hypertrophy. Furthermore, the sizes of the antra showed a 65% increase over those in the control right ovary, yet this increase was not as great as that in the operated control opossums where the compensatory hypertrophy of antra size was as much as 150%. This would indicate that the androgenic treatment in this case was not given in sufficiently large dosage and/or did not extend over the length of time necessary to stimulate or inhibit the ovary.

Treatments of 1 mg. of t-propionate for 20 days caused a 20.9% decrease in gross weight over the control right ovary and an approximate 85% decrease over the untreated controls manifesting the compensatory hypertrophy. This would indicate that at this dosage and length of treatment there occurred a definite decrease in size of the ovary due to the androgenic substances. Measurements of the follicular antra were about the same as those in the control ovary, but there was an increase in the number of degenerating follicles and of ova undergoing atresia (fig. 19). The results of the higher dosage of 5 mg. of t-propionate each day for 20 days compared with those of the 1-mg. dosage. The reproductive tract was stimulated by the androgens and the weight increased, in general, directly as the concentration of androgen increased. After treatment, the primary tubules (cf. fig. 19) and germinal epithelium were similar to those of the controls. Only one case existed in which the rete of the androgen-treated ovary was larger than that of the control, and since this was not consistently the case the results were questionable.

In resumé, it may be pointed out that androgenic treatment with the dosage employed, failed to affect the ovary during development up to day 100; in the juvenile and adult ovary, however, such treatment rather consistently led to reduction

in ovarian weights and to interference with follicular development.

THE EFFECTS OF ESTROGENS ON THE OVARY

To determine any possible influence of estrogenic hormones on the developing ovary, juvenile or adult stages, weights were taken whenever it was possible to dissect out the organ, but otherwise planimetric determinations of volumes were made. All ovaries were serially sectioned and studied histologically. The estrogenic substances were found to be very toxic to the pouch-young and many fatalities resulted. Estrogenic hormones were administered to the pouch-young mainly by inunction in ointment form starting as early as day 5. In some cases, subcutaneous injections of the estrogenic hormones in oil solution were given. The ointment contained 0.625 mg. of estradiol per ounce; its estrogenic properties had been tested by Moore, Lamar, and Beck ('38). It is difficult to say just how much estrogen was absorbed by this method because of the increasing surface area of the young as they grew, and because of the contact of the young with the mother's pouch. The approximate amounts of estradiol applied per day for the younger stages were in the order of 1 γ , while in older stages of the pouch-young the amount applied was not over 6 γ .

Subcutaneous injections into the pouch-young, juveniles, and adults were made in oil solution containing Progynon B, which is the benzoic acid ester of the dihydro follicular hormone, estradiol. The concentrations of this hormone used in the pouch-young were from 20 IU to 125 IU per daily dose, while in the juvenile and adult anestrus stages the daily dosage was from 50 IU to 100 IU. Estrogen-treated ovaries of twelve pouch-young and four juvenile and anestrus adults were compared to adequate normal controls. Sample data of some of these are listed in tables 1 and 2.

Estrogenic treatment up to 100 days. The volumes of the ovaries, determined by planimeter, were neither significantly stimulated nor inhibited by estrogenic hormones in any of the opossums of this earlier age. The cortical area, germinal

epithelium, size and appearance of primary tubules, and the size and number of germ cells were similar to the untreated control animals, thus evincing neither stimulation nor inhibition. Determinations of size were made by averages of ocular micrometric measurements. The rete in the ovary was questionably stimulated up to 44 days after which there was no apparent effect upon it. The androgens stimulated the rete slightly more than did the estrogens. The epoöphoron tubules, as with androgens, were stimulated considerably by the estrogens; average diameters of the tubules of treated specimens at day 16 were approximately double those of controls; whereas on day 24 they were $9\times$, and on day 46, $21\times$ normal.

The gross size of the mesonephros was increased by the estrogens more than by the androgens; the increase was accounted for by an edema of the connective tissue and some hyperplasia. The mesonephric tubules were dilated, but the cells of the epithelial lining were low.

Estrogenic effects on juveniles. Two opossums at day 150, when treated with 100 IU of the estrogen Progynon B each day for 12 days, showed a varied response (table 1). The ovary of treated opossum 170 did not differ significantly in weight or in histology from the untreated controls, but treated opossum 171 with the same estrogenic treatments showed a 30% decrease in weight, decrease in number of follicles and atretic ova. Thus, the ovary at day 150 showed decreased weight in one case and no apparent effect in the other. The average weights of the reproductive tracts of the treated were $8.9\times$ the average weights of the untreated controls.

The ovary of opossum 170 exhibited three accessory nodules on the side of the ovary, composed of tunica albuginea and secondary cords still attached to a very active germinal epithelium. Secondary cords had already been broken up in normal ovaries at this age. These growths from the side of the ovary were connected to it by a stalk near the hilus. Such structures were first well described by Beigel, in 1877, on 8 out of 350 cadavers. Beigel believed they functioned like primary ovaries and in short were the same. Hartman ('27)

had also observed the "accessory ovaries" of Beigel in opossums.

Estrogenic effects on the anestrous ovary. Two animals were treated daily with 100 IU and 50 IU of Progynon B for 12 days, causing a significant decrease in weight of the ovaries as compared to the weight of the control ovary removed before treatment. (For details of the operative procedure see androgenic effects on the anestrous opossum.) The protocols of the estrogenic treatments are listed in table 2. The decrease in ovarian weight was only 14% for a daily dosage of 100 IU and 31% for 50 IU, when compared with the weight of the control right ovary removed before treatment. These percentages of decrease appear low but become more significant after consideration of the left ovary which averaged a 63.7% compensatory hypertrophy upon unilateral ovariectomy (table 2, serials 2(10)A and 3(11)a.) The treatment not only prevented compensatory hypertrophy but also led to an additional 14% to 31% decrease in weight from the normal ovary. Compared with operated untreated ovaries the decreases of treated ovaries were in the order of 75% and 95%.

Histologically the treated ovary showed almost a complete lack of antra in the follicles and a decrease in number of primary follicles (cf. fig. 6). Remains of many degenerated follicles were manifested by large lightly staining cells and debris filling many areas. The estrogens did not appear to speed up the process of germ cell formation, nor to affect the size of the primary tubules (fig. 6). The rete was enlarged slightly in one treated ovary, but within the normal range of variation.

In summary, it has been shown that the estrogenic treatments failed to modify the ovary up to day 100, but on the juvenile and adult ovary, such treatments led to reduction in ovarian weight, and to damage of the follicles and germ cells.

THE EFFECTS OF GONADOTROPIC SUBSTANCES

Only brief mention will be given to the effects of gonadotropins on the developing opossum ovary since further studies are in progress and will be reported later.

Prior to day 100, five females treated with gonadin were studied; these were autopsied on days 17, 21, 30, 38, and 100, subsequent to daily treatments in concentrations of 1-5 ru per injection; treatments extending from 10-15 days. Volumetric determinations of ovaries were made in four cases and are included in text figure 1. It will be seen that the treatments did not perceptibly modify the volume of the ovaries and histological studies failed to reveal any significant change from the normal. Studies of the female reproductive tract failed to show modifications that suggested additional estrogens. Hence, it is believed that the ovary does not respond to gonadotropins up to an age of approximately 100 days; evidence is already in hand to indicate responses at slightly older stages.

PLANIMETRIC ANALYSIS OF VOLUMES

Though there seemed to be no evidence from histological studies of either stimulation or inhibition of the ovaries of pouch-young opossums after hormonal treatments, it was not possible by microscopic observations to know definitely whether there had been hypertrophy or atrophy of the ovary. The ovaries were not always the same shape, nor was it always possible to section them in just the same plane. Great difficulty was experienced in weighing the ovaries from these very small opossums and in making gravimetric comparisons. Sections of the ovary were projected at 167 diameters and from planimetric measurements the relative volumes of the ovaries were determined; these planimetric determinations, showing variations of less than 0.5% in three measurements, are recorded in table 1. The results of the planimetric analyses were plotted graphically and analyzed statistically (text fig. 1). The age of the pouch young was represented along the abscissa and the planimetric volume units, along the ordinate.

Determinations were made to see whether there were any statistical differences within the four series — the normal; androgen, estrogen, and gonadotropin treated. The differences were expected to be less in the younger animals than in the

older ones because of the greater absolute size of the latter. Thus, it was necessary to consider percentage rather than absolute differences. One animal of each of the four groups was studied at 16 or 17 days and similarly one of each group at 30 or 31 days. In these cases it was necessary merely to find the percentage differences from the averages of the two age groups. Above this, the ages varied and it became necessary to estimate the average trend from which the percentage of deviation might be taken. It was obvious from inspection, that the observations from 38 days on were clustered reasonably well along a straight line and that they were not numerous enough to justify any attempt at a more refined description

TABLE 3
The average percentage of deviation ($\bar{d}$) from average trend compared with the standard error.

	$\bar{d}$ STANDARD ERROR
Normal	+ 2.0 $\pm$ 10.9
Androgen	— 7.0 $\pm$ 12.0
Estrogen	— 5.9 $\pm$ 15.5
Gonadotropin	+ 10.2 $\pm$ 13.4

of the trend. The straight line fitting these ten observations best was accordingly found by use of the formula of least squares. When x represents age in days and y , the volume of the ovary in appropriate units, the slope of the line was found by the formula, $b_{yx} = (\Sigma xy - \frac{\Sigma x \Sigma y}{10}) / (\Sigma x^2 - (\Sigma x)^2 / 10)$. The result was 1.096 volume units per day. The mean age of the ten was 67.2 days and the mean volume was 52.8 units.

The formula for the estimated average, y_c , at any age, x , is $y_c = \bar{y} + b_{yx} (x - \bar{x})$ which reduces to $y_c = 1.096 - 20.86$. The percentage deviation, d , of each observed volume from the trend value, y_c was then obtained. The average percentage deviation, $\bar{d}$, was found for each of the four series (table 3). A single standard deviation of percentage differences was found for the eighteen observations by the formula $\sigma^2 d = \Sigma d^2 / 14$. This formula made allowance for the

loss of 4 degrees of freedom in calculating the mean at age 16 or 17, at age 30 or 31, and the mean and slope for ages 38 to 100. No allowance was made, however, for possible real differences among the series. The standard error for each series average was found from the formula, $S.E. = \sqrt{d^2/n}$ where n was the number of observations in the series in question.

In no case was the average deviation as great as its standard error (cf. table 3). One must conclude, therefore, that in this set of data neither estrogen, androgen, nor gonadotropin, when applied to opossum pouch-young up to 100 days, stimulated or inhibited the volume of the developing ovary. These data compare favorably with the planimetric determinations made on the testis (Moore and Morgan, '42).

DISCUSSION

A. Normal development

The study of normal development of the opossum ovary was necessary for the careful analysis of any possible modification of it by hormones. Such a study of normal embryology has shown that, for the most part, the ovary followed the usual mammalian course of development but with many features of special interest. Particular attention is to be directed to the presence of primary sex cords and tubules containing developing ova throughout ovarian development and the retention of primary tubules in the adult ovary; the presence of cell nests in the developing ovary with evidence suggesting that stromal cells broke up these nests and differentiated into granulosa cells; the formation of rete from evagination of the peritoneum of the rete region of the genital ridge and its subsequent connection to the evaginations from Bowman's capsules with eventual growth into the ovary.

The germinal epithelium. The germinal epithelium in the opossum had two distinct phases of germ cell activity. The first, present at birth, was the proliferations of the primary sex cords; the second, the proliferations of oögonia of the

cortical region throughout life, including, the formation of secondary sex cords beginning at day 5, and the continued formation of ova singly and by small strands of epithelial cells. Some of the mesenchyme cells of the ovary appeared to have originated from the germinal epithelium.

In most cases in juvenile and adult stages, the germinal epithelium was especially active near the hilus of the ovary; this was not consistently the case since the activity was greater in some ovaries far from the hilus. Also, in various ovaries of the pouch-young there was the suggestion of increased activity near the hilus, so that secondary cords with open lumina were formed. Hartman ('26) reported briefly that the germinal epithelium in the adult opossum was more active at the hilus than elsewhere, and the germ cells were not produced singly, but from strands of epithelial cells proliferated from the germinal epithelium. Everett ('42) observed the formation of the ova in the adult opossum and stated that the germinal epithelium produced germ cells singly and was active more often near the hilus. Simkins ('28) maintained that after the proliferation of germ cells, which later formed the secondary sex cords, the germinal epithelium of the human ovary retrogressed, became inactive and resembled coelomic epithelium.

The continual proliferation of the germ cells from the germinal epithelium throughout ontogeny of the opossum, however, agreed with the ideas of most investigators, such as Arai ('20) and Brambell ('27) on the mouse, Allen ('23), Butcher ('27) and Hargitt ('30 b and c) on the rat, and Evans and Swezy ('31) on other mammalian ovaries. However, Kingsbury ('13) and Winiwarter ('00), in the cat and rabbit respectively, believed ova were not produced after birth, but Winiwarter and Sainmont ('08) in the cat and Kingery ('17) in the mouse thought an activity of the germinal epithelium existed until puberty and that ova formed just before sexual maturity persisted as the definitive ova, while the earlier ova degenerated. A few of the germ cells in the opossum that developed just before sexual maturity persisted a short time in the mature ovary. There were periods in the early develop-

ment of the opossum ovary when the germ cells were formed rhythmically in greater numbers. Both Sneider ('40) and Duke ('41) reported rhythmic formation of the ova in the rat, cat and rabbit.

The two distinct proliferations from the germinal epithelium in the opossum, previously mentioned, were separated rather sharply by the primary tunica albuginea which had made its appearance by day one and was visible up to 67–100 days of age. In the kitten the secondary cords were connected to the primary cords as late as 16 days postpartum (Winiwarter and Sainmont, '08). The primary cords in the chick (Swift, '15), pig and rabbit (Allen, '04) were separated by the intervening connective tissue layer, the tunica albuginea. A distinct basement membrane under the germinal epithelium was not detectible in the opossum ovarian development.

The primary cords. Primary cords were present at birth in the gonad and then either completely disappeared in the ovary by day 16 or continued to persist in variable amounts throughout the adult period. Germ cells in primary cords in early development increased in size until the walls of the cords were stretched and the entire structure appeared similar to a cortical follicle, and thus the medullary follicle was formed (figs. 13, 18, and 20). In adult ovaries, however, the primary tubules were much larger but the germ cells failed to increase proportionally. As a result the primary tubules were not stretched into follicular form by the developing ovum, and a typical cumulous oöphorus did not surround the ovum. Hence, the cells of the primary tubules may not be typical granulosa cells; the lack of antra also supports this interpretation. The tubules were not always connected and many short fragmentary tubules were located throughout the cortex and medulla. These fragmentary tubules appeared to arise from the action of the ovarian stroma on originally longer cords.

Discussions of medullary follicles and primary cords of the ovary are scarce in the literature and those available are conflicting. Observations of primary cords and medullary follicles in the developing ovary were first mentioned by Wini-

warter ('00) on the rabbit. Later Sainmont ('05), on the cat ovary, believed medullary follicles were derived from the secondary cords. Following this report much attention was given to the medullary follicles by Winiwarter and Sainmont ('08) on the kitten ovary. They observed primary cords in the early developing ovary but after the secondary cords had made their appearance, the two sets of cords became connected and remained so until 16 days postpartum. Ovules existed in these primary cords, but for unknown reasons they were interpreted as being just hypertrophied indifferent cells which subsequently returned to normal size. Thus they conformed to the definite cell lineage theory. Kingsbury ('13) gave much attention to the medullary follicles in the morphogenesis of the cat ovary. He proposed that the medullary follicles, consisting of cords and masses of cells within the medulla, which in some cases connected to the primary cords, were derived in part from the secondary cords of the embryo and that the ovules in the medullary follicles were derived from the secondary cords at the time of their connection; the connection existed at the time the absence of the primary tunica albuginea was noted. He stated that the term "medullary follicle" had significance only as a descriptive term of location and had nothing to do with being formed from the true primary cords.

More recently League and Hartman ('25) observed medullary follicles in the opossum, *Macacus* (monkey), armadillo, and dog. They called these "anovular follicles," although in some cases they showed figures of the follicles containing ova; they considered these follicles to be derivatives of the secondary cords. Our studies suggest a modification of the opinion of League and Hartman. In the opossum it is suggested that the ova in the medullary follicles arise by differentiations within primary cords, and not within secondary, since a primary tunica albuginea exists from the very beginning separating the primary and secondary cords in early ovarian development; furthermore, the observation of ova developing in the primary cords and the lack of evidence for any migra-

tion of cells from the secondary cords into the primary cords substantiates this point of view.

Ovaries manifesting some of the testicular homologues along with the normal components are not unusual in lower vertebrates. The ascidians and the lower vertebrate lamprey show hermaphroditism. Okkelberg ('21) has shown that during a more or less extended phase of juvenile hermaphroditism in the lamprey, the oöcytes and spermatocytes differentiate side by side. In sex intergrades of urodeles, reptiles, birds, and mammals, ovarian and testicular tissue have been observed to exist mutually side by side without any apparent antagonism. Willier ('21) described certain freemartin gonads in which ovarian portions with distinct follicles containing germ cells coexisted with testicular portions containing typical seminiferous tubules lacking germ cells; however, in some foetal freemartin gonads, female type germ cells were found in primary cords (this was suggestive of some normal opossum ovaries described above); these germ cells degenerated before birth. The presence of the ovarian part, which is apparently an exceptional condition in the freemartin gonad, was thought by Willier to be the result of a late introduction of the male sex hormones. It was pointed out by Witschi ('39) that in the rare hermaphroditic mammalian gonads, ovarian and testicular tissue, in many cases, did not compose separate organs, and that the testicular part did not always bear male germ cells, though sometimes the testicular tissue was in the scrotum and the ovarian portion did produce normal female germ cells.

There appears to be no sharp line of demarcation determining whether the gonad is ovary or testis, but a long line of gradual transition upon which the center could be represented as the intersexual condition and the extremities the most typical ovary or testis. Nearly every adult opossum ovary examined had a variable amount of active primary tubules present; perhaps the ovaries with the most primary tubules more nearly approached the intersex condition. Sex of the gonad could then be a quantitative rather than a qualitative

difference with the genes governing the process through some physiological mechanism. This evidence may then suggest a very simple interpretation of the occasional ova-testis in hermaphrodites.

As stated by Witschi ('14, '29) the germ cell depends on its tissue environment for determination into either ovum or sperm. If found in the medulla, it is to be a sperm and if in the cortex, an ovum. In the opossum this notion did not strictly apply. Medullary tubules gave rise to germ cells that grew in a manner entirely similar to those derived from secondary cords. When the size of the cell became sufficiently great to distend the wall of the primary tubule a structure evolved that was very similar to a typical follicle derived from cortical sources; instead of stromal cells, producing granulosa, the surrounding cells were just medullary cord cells. Occasionally in the adult ovary these medullary germ cells were still present but not in follicular form.

The secondary cords. According to most recent observations the secondary sex cords arise from the germinal epithelium in mammals, as do the primary cords, with only a difference in timing. However, a few modifications of this type of development are mentioned in the literature. Kingsbury ('13) refers to the egg tubes of Pflüger (secondary sex cords) in the cat as cell trails left in wake of the advance of the surface of the ovary as the ovary increases in size. In the opossum it was found that the greatest increase in size of the ovary occurred after the secondary cords were formed by invaginations and down-growth; i.e., cords were found from 5 to 31 days, but the greatest increase of ovarian size was from 31–44 days, when cells of the secondary cords were rapidly growing oöcytes. In the human (Simkins, '28), cells are proliferated from the germinal epithelium. These cells later became arranged in clusters forming the incipient secondary cords.

Cell nests, made up of enlarged germ cells, appeared in the secondary cords of the opossum at 44 days and were finally broken up by day 150. Hargitt ('30 a) referred to epithelial cells in nests in the ovary of the rat embryo at day 17, to day

3 postpartum and maintained that only stroma of fibrous tissue was about each nest. The nests were finally broken up by the stromal cells. In the rat, these nests were made up of three kinds of nuclei; irregular or elongated, which later formed the follicle cells (how this change occurred was not explained clearly), and large and small spherical nuclei.

In the opossum there appeared to be only one kind of cell in the nests and it was the enlarged germ cell. Subsequently the nests were broken up by the action of the surrounding stromal cells penetrating the nests and then separating the germ cells and producing primary follicles (cf. figs. 5 and 14). Since all stages of differentiation were seen between the stromal and granulosa cells, it was suggested granulosa cells were formed from mesenchyme cells under a possible inductive influence from the germ cell. Follicular formation outside the nests and in mature ovaries appeared to follow the same kind of development. The single ovum was surrounded by stromal cells and the differentiation of the granulosum cell was similar to that already described. A single definite granulosum cell in the stromal tissue was never seen. However, Allen ('23) and other investigators have stated that granulosa cells have been derived directly from epithelial cells from the germinal epithelium, since the follicular cells were closely associated with differentiating ova before and after they sank into the stroma. Our interpretation is only a modification of this, since we have suggested granulosa cells may differentiate from connective tissue stromal cells rather than from epithelial cells, but the stromal cells may have had their origin either from the germinal epithelium or migrations from the mesovarial region. The granulosa cells, in general, degenerate progressively after follicular atresia. Thecal cells in the opossum follow in general the same trend of development and fate, and in formation of corpora lutea, both thecal and granulosa cells produce similar types of luteal cells.

Rete cords. Authors have expressed three diverse opinions concerning the origin of the rete cords. Braun (1877), and other later writers, have asserted that in the amniotes they

are formed from the epithelium of Bowman's capsule. Some claim them to be derivatives of the mesenchyme situated between the germinal epithelium and the glomeruli of the Wolffian body (Swift, '15, for the chicken and Wichmann, '12, for the pig and dog). The third opinion is that they come from the peritoneum as Allen ('04) has described for the pig. The latter type of development is the one observed in the opossum and Burns ('41) has recently expressed the same view. The presence of a cavity in the rete of mammalian ovaries was noted by Wilkerson ('23) for ovaries of man, cow, pig, and dog. The opossum ovary can be added to this list. In the female, connections between the rete and primary sex cords were never made in the pig (Allen, '04), or human (Simkins, '28) or the union was imperfect in the human (Wilson, '26), but in the calf, connections were made only in the freemartin ovaries (Willier, '21). In the opossum, connections between the rete and primary cords were of usual occurrence in developing ovaries and were maintained in some adult ovaries (cf. figs. 8, 13, and 18).

B. Hormonal treatments

The study of the effects of hormones on the morphology of the ovary from birth to maturity has revealed, among other things, several aspects of essential interest, such as: 1. Failure of the hormones to modify the normal method or rate of ovarian "embryonic" differentiation. 2. Failure of androgens to produce the freemartin testis-like gonad. 3. Appearance of first ovarian response to hormones at about day 125-150. 4. Observation of some ovarian activity during the non-breeding anestrus season. 5. The response of the anestrus ovary to hormonal substances.

The undifferentiated gonad through sexual differentiation. Burnes ('39 d, e) believed there was a distinct reduction in the gross size of the ovaries of the pouch-young opossums after treatments with the androgens and estrogens, though he did not list the measurements or weights of the ovaries to substantiate this statement. His somewhat greater dosages may

have been responsible for this slight difference from our own observations. He reported that the rete was expanded into a loose saccular space which may have had contact with the sex cords in the animals treated with t-propionate, whereas in the normal the sex cords had no contact with the rete. He explained the reduction of ovarian size on the basis of pituitary inhibition by the hormone according the hormonal interrelationship between the gonad and hypophysis as developed by Moore and Price ('32). Our fragmentary results from gonadotropic treatments, however, indicated that the ovary was refractory to gonadotropins during early pre-juvenile stages which would suggest that the pituitary, even if inhibited, would have had little effect on the ovary. In this study the rete in the normal ovary was found to contact the primary cords by the sixteenth day, and by the thirty-first day connections were observed. Our observations agree with the report of Burns that androgens or estrogens produced no apparent change in the histology of the tissue of the gonad.

Turner ('39) found that the administration of large amounts of androgens during the intrauterine development of the mouse neither prevented the differentiation of the ovarian cortex nor induced hypertrophy of the ovarian medulla. Likewise Green, Burrill and Ivy ('39 a, b, '40), on the rat, and Raynaud ('37, '38 a, b, c, '39), on the mouse, have found virtually no effect on the development of the ovary from the application of estrogenic and androgenic hormones to the mother during pregnancy. Dantchakoff ('36) reported that the administration of androgens to guinea pig embryos, by way of the amniotic cavity, resulted in an ovary provided with a plentiful cortex.

On the fish, *Xiphophorus*, Regnier ('38) asserted that t-propionate reduced the ovaries of true females, but that sex reversal occurred in only those that were predetermined to change into males.

In amphibians, with the administration of androgens, the ovary was gradually transformed into testis, or testis-like organ in *Rana temporaria* (Gallien, '38), in *Amblystoma*

punctatum (Burns, '39 a) and in *Rana clamitans* (Foote and Witschi, '39). When the androgen was accompanied by pituitary substances to hasten the slowly developing gonad in *Rana catesbiana* (Puckett, '40), the results were similar.

For reptiles, the results of Forbes ('38, '39) on juvenile alligators, and Risley ('41) on juvenile terrapins indicated that t-propionate had little effect on the ovary with the exception of a decreased follicular size. Risley also reported that with estradiol dipropionate the ovary was relatively uninfluenced, and with gonadotropic substances the ovary was stimulated to increase in size and to secrete substances to which the accessory sex organs responded (similarly Forbes, '37, on juvenile alligators). They concluded that the ovaries of reptiles responded to pituitary hormones earlier than those of the mammals.

In the chick, androsterone injected into the developing eggs, caused the ovary of the chick to be converted into an intersexual state (Wolff, '35). However, Willier and coworkers ('37) failed to note any marked effects on the ovary from male urine extract, but later ('38) it was found that androsterone did exert stimulating effects upon the medulla and exercised some cortical suppression of the left ovary; he failed to find any effect on the ovary after estrogenic treatment.

In resumé, the effects of estrogens, androgens and gonadotropins on the ovary, from undifferentiated gonad through the stage of sexual differentiation, appear to have been negligible in modifying the rate of growth or method of differentiation in the mammals. In groups below the mammals, some modification of the developing ovary did occur after the use of hormones.

Juvenile and adult mammalian ovaries. Ovarian atrophy was obtained with testosterone in the rat by some investigators (Fisher, '38; McEuen and coworkers, '37), while stimulation of the ovary and formation of corpora lutea was produced with the same treatment by others (Nelson and Merkel, '37; Hamilton and Wolfe, '38; McKeown and Zuckerman, '37). Moore and Price ('30, '32) suggested the decrease

in weight was caused by the inhibition of the anterior lobe of the pituitary, while Korenchevsky and Hall ('40) believed that it was a toxic action of the hormone resulting only after treatments of long duration and the effect of short duration treatments was to exert a direct stimulation on the ovary. Others, Nathanson and coworker ('38) and Fluhmann ('41), explained that the administered sex hormones acted upon the anterior pituitary to stimulate secretions that induced the formation of follicles and corpus luteum. Fluhman failed to elicit ovarian responses in hypophysectomized females injected with sex hormones.

Large doses of estrogens for a short time (around 14 days) in the rat (Hohlweg, '34; Wolfe, '35; Ellison and Burch, '36; Mazer and coworkers, '36) and in the mouse (Burdick and Whitney, '37) led to formation of corpora lutea. With continued administration in the rat of large doses (Selye, '35) or chronic small doses (Calatroni, '34), and in the mouse of continued large or small doses (Gardner and Allen, '37), the corpora lutea involute. The question of whether the gonadotropic effects of the steroids are due to the direct action on the ovary or indirect action of the pituitary was discussed by Selye and Collip ('36). They stated that the steroid could exert a direct gonadotropic effect, for in hypophysectomized rats, the ovaries became atrophic and could not be stimulated by estrone; but if this atrophy was prevented by maintenance doses of pituitary gonadotropic substances, estrone had a direct effect on the ovaries, since it produced "pregnancy-type" corpora.

In summary of the above controversial results from the use of estrogens and androgens, it seems that the problem was not settled. The ovary apparently reacted differently to these steroids according to the four variables; the concentration of hormone, the age of the animal, the length of time the injections were given, and the species of animal involved in the experiment. Androgenic-treated anestrus adult opossums reacted to small doses and large doses given over a long period (about 20 days), by atrophy of the follicles and of the

entire ovary. As the result of small doses over a short period (about 12 days), there was no inhibition and apparently no significant stimulation. Reacting to both small and large doses of estrogens over a short period, the anestrus ovary was atrophic, but to large doses over a short period the ovary of one juvenile opossum was atrophic and another juvenile gave no response. Whether this reduction in ovarian size in the opossum after the steroid hormonal treatments is due to direct toxic action on the ovary or to inhibition of the pituitary as suggested by Moore and Price ('30, '32) cannot be answered from our data. However, in our experiments the concentrations of steroid hormones used had no direct stimulating effect on the ovary during the times over which treatments extended.

The mammalian ovary responds definitely to gonadotropic substances as pointed out by Smith ('26), but he showed clearly that rats younger than 20 days responded less readily. Wiesner ('32) demonstrated that ovaries of 2-day old rats failed to respond to gonadotropic substances but gave definite responses between ages of 4 to 10 days. In the opossum a similar refractoriness of ovaries to gonadotropic stimulation appeared to hold since results thus far obtained have revealed ovarian responses only after 100 days of age.

Comparison of freemartin gonad with ovary treated with sex hormones. When these observations of opossum ovarian development under the influence of chemically isolated hormones are brought to bear upon the conditions that were inherent in the freemartin (Lillie, '16, '17), it should be recalled that definite ovarian modification was induced in the freemartin female twin by humoral substances presumably of testicular origin. These blood-borne substances effectively modified the developing ovary toward a testis. Most secondary cords were caused to degenerate or to be inhibited in their development; medullary cords frequently developed into structures similar to seminiferous tubules; the rete connections to the seminiferous tubules and to the mesonephric tubules persisted to complete an excurrent system similar to

that in the male (Willier, '21). The great amount of variation found in freemartin ovaries was presumably due to variation in time of onset and to the amount of substance brought into the female. Hughes ('27, '29) described a freemartin condition which occurred in monochorionic twins of pigs. The ovary was modified, but not to the extent that it was in the freemartin described by Lillie, since the germinal epithelium did not completely lose its activity. Cases of monochorial twins occurred regularly in the marmoset (Wislocki, '32) with visible anastomoses of umbilical veins. The gonads were characteristic ovaries and testes, with no modification to a freemartin condition. Typical freemartin conditions have only been reported, therefore, among the mammals in the artiodactyls.

Chemical hormones have failed to exert a marked influence on ovarian development in the opossum. No interference was noted on secondary cord development and the ovary proceeded in its differentiation in an essentially normal manner. Since chemical androgens have failed to modify ovarian development in the opossum when applied directly to the developing young, and have failed to modify ovaries of other mammals developing in the uteri of mothers receiving treatment with androgens, it becomes evident that these treatments either do not duplicate the conditions effective in the freemartin or that there is a great difference in reactions of species to similar conditions.

Embryonic humoral substances. Since the chemical hormones have failed to modify the differentiation of the developing ovary, the question is raised as to whether there is evidence for humoral substances which will modify the differentiation of developing ovaries. Although such a hormone has not been isolated, two excellent cases have been reported which substantiate the opinion that such hormones do exist in at least two species; the one, the freemartin which has already been discussed, the other reported by Witschi ('31) in the frog. These substances may not be the same since the former was demonstrated to be blood-borne while the latter was demonstrated to be diffusible through the tissues. Witschi's

explanation for sex determination in frogs was placed on certain genes which were responsible for emanations of some sort sent out by the cortex and medulla and spread out over the embryo. If the substance was from the cortex it inhibited the medulla and vice versa. The conditions as listed by Witschi, in general, appeared similar to the mammalian sexual differentiation but not identical to it. Witschi's explanation should be given careful consideration.

The effects of hormones on germ cells. The early development of the germ cells did not appear to be influenced directly by the administration of hormones when applied by ointment on the surface of the skin or injected subcutaneously. Careful measurements were made of the ten largest germ cells of each ovary and the averages compared. It seemed that the germ cell differentiated independent of the hormones used in our experiments. Similar results were obtained in hypophysectomized animals. Abortive polar body formation took place for several months after hypophysectomy in rats (Smith, '30), and McPhail ('35) found that in cats, and probably in other mammals, the ova grew to a normal size after hypophysectomy. This may be further evidence that it is perhaps not necessary for early formation of germ cells in the developing embryonic ovary to be dependent upon the hypophysis. Stein and Allen ('42) have shown, however, that estrogens injected into the capsule around rat ovaries caused increased proliferation of the germinal epithelium. More work needs to be done along this line to answer definitely the question of germ cell stimulation by hormonal treatments.

The effects of hormones on the Wolffian body. The steroid hormones appear to exert a greater effect on the Wolffian body and its components during "embryonic" development than on the derivatives of the genital ridge. The epithelial lining and the diameter of the tubules of the mesonephros were hypertrophic after treatment with t-propionate and gonadotropins. Whether the effects were the results of a direct localized stimulation of the tubules or of a general metabolic change, is not known. Selye ('39) reports that testosterone

caused a hypertrophy of the cells of the epithelial lining of the proximal and distal convoluted tubules of the kidney in rats. Albert ('42) verifies this report but in addition mentions that in rats treated with estradiol the tubules were narrow and lined with a low epithelium. In the opossum after treatment with estrogens, the mesonephric tubules were lined with a low epithelium, but were also dilated. The results from hormonal treatments on the mesonephros thus appear similar to those on the kidney.

SUMMARY AND CONCLUSIONS

A. Normal development of the ovary

1. The opossum gonad is indifferent at birth but has primary cords. It is detectibly ovarian in 3-5 days and has intense cortical development between days 5 and 100, well developed follicles without antra from day 44 to past 100 days, and oöcyte nests between days 44-125 which undergo changes by degeneration and by follicle formation.

2. Ova originate in the primary cords and rhythmically from the germinal epithelium. Most ova developing in the ovary before the breeding stages degenerate and are never ovulated. Ovarian stroma may also differentiate from the peripheral germinal epithelium.

3. Granulosa and thecal cells may differentiate from the stromal mesenchyme. The stroma does not play a passive role in the ovary.

4. Primary cords or tubules, which may contain ova that always degenerate, are found in most ovaries throughout ontogeny; these tubules, in some cases, connect to the rete tubules. Medullary follicles are formed in early ovarian development.

5. Growth of the ovary is most rapid from days 31-44, corresponding to growth of the cells in the "cell nests" after differentiation into oöcytes.

6. Anestrus prevails in adults from June to February in the Chicago region, but the ovary is not completely inactive.

B. Normal development of the rete

1. The rete originates from the mesothelium of the rete ridge, may branch to connect short evaginations from the epithelial wall of several of Bowman's capsules, then all rete join into a common duct which immediately goes into the ovary where it again branches. After the lumen is formed, it is maintained throughout the ontogeny of the opossum. The connection of the rete to the peritoneum may or may not be lost.

2. The epoöphoron tubules connect with a lumen to the rete as in the male opossum, and thus the functional connections of the testis to the Wolffian duct is duplicated in pouch-young opossums.

C. Effects of hormones on the ovary up to 100 days

1. Microscopic studies and planimetric analyses of volumes show that the ovaries of pouch-young opossums are neither stimulated nor inhibited by the sex hormones (androgen and estrogen) or gonadotropic hormones up to 100 days.

2. There are no freemartin effects.

3. Since the ovary did not respond to gonadotropic substances until relatively late stages it is suggested that the ovary does not secrete sex hormones of an adult type during its early stages of embryonic differentiation.

4. Germ cells appear to differentiate independent of the hormones used. The rete is questionably stimulated by the estrogens and only slightly stimulated by androgens from day 16 to day 44, but does not respond significantly at later periods.

D. Effects of estrogens on juveniles and anestrus adults

1. The ovaries respond by a decrease in weight, fewer follicles, and by increased atresia of follicles and germ cells. The size of the primary cords is not modified, but there is some atresia of the germ cells contained within.

E. Effects of androgens on juveniles and anestrous adults

1. The ovaries of juveniles are decreased in weight and the follicles undergo atresia. In the anestrous adult, the small dosage of androgens affects neither the weight of the ovary nor the size of the follicles significantly. With large dosages of androgen there is a decrease in gross weight and an increase in follicular atresia, but the size of the antra are little affected. (The effects of estrogens appear to be more drastic than those of the androgens; this, however, may be due to size of dosage.)

F. Effects of hormones on the mesonephros

1. The connective tissue shows hyperplasia with androgens, hyperplasia plus edema with the estrogens, and only a slight hyperplasia with gonadin.

2. The mesonephric tubules show a "renotrophic" effect from androgens and gonadin, with high epithelial lining and dilated tubules, but with estrogens there is a low epithelium with dilated tubules.

G.

Since the adult hormones seem to play a doubtful part in gonadal development, various working hypotheses have been discussed.

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EXPLANATION OF PLATES

All of the figures are photomicrographs from opossum serial transections

ABBREVIATIONS

AF, atretic follicle	Oc, oöcyte
CN, cell nest	Ov, ovary
BC, Bowman's capsule	PC, primary cord
DTA, definitive tunica albuginea	PF, primary follicle
DO, degenerating oöcyte	PT, primary tubule
Ep, epoöphoron	P'TA, primary tunica albuginea
ER, evagination of rete	PrF, primordial follicle
GE, germinal epithelium	R, rete
GF, graafian follicle	RF, reticular fiber
IR, invagination of rete	SC, secondary cord
MF, medullary follicle	StC, stromal cell
OSC, open lumen secondary cord	WD, Wolffian duct

PLATE 1

EXPLANATION OF FIGURES

Ovary of Opossum

1 Normal, age 1 day (23), showing primary cords in gonadal portion of genital ridge and relationship to Bowman's capsule. 233 X.

2 Normal, age 5 days (1). Ovary and portion of mesonephros. 44 X.

3 Normal, age 67 days (83 B). Cell nests shown with primordial follicles formed from them. Rete connected with primary tubules. 44 X.

4 Normal, age 31 days (18). Secondary cords shown with many oögonia. Oöcyte leaving germinal epithelium in upper right corner. No cell nests; compare with figure 17. 233 X.

5 Normal, age 100 days (82 B). Showing a method of formation of primordial follicles and fate of oöcyte nests; stromal cell moving in around oöcyte (cf. fig. 14); many primordial follicles, upper left, formed from cell nests; primary follicle, lower center, and oöcyte leaving germinal epithelium, upper right corner. 180 X.

6 Estrogen-treated adult during anestrous season (B 5). Note scarcity of follicles, normal appearance of primary tubule and rete tubules. 44 X.

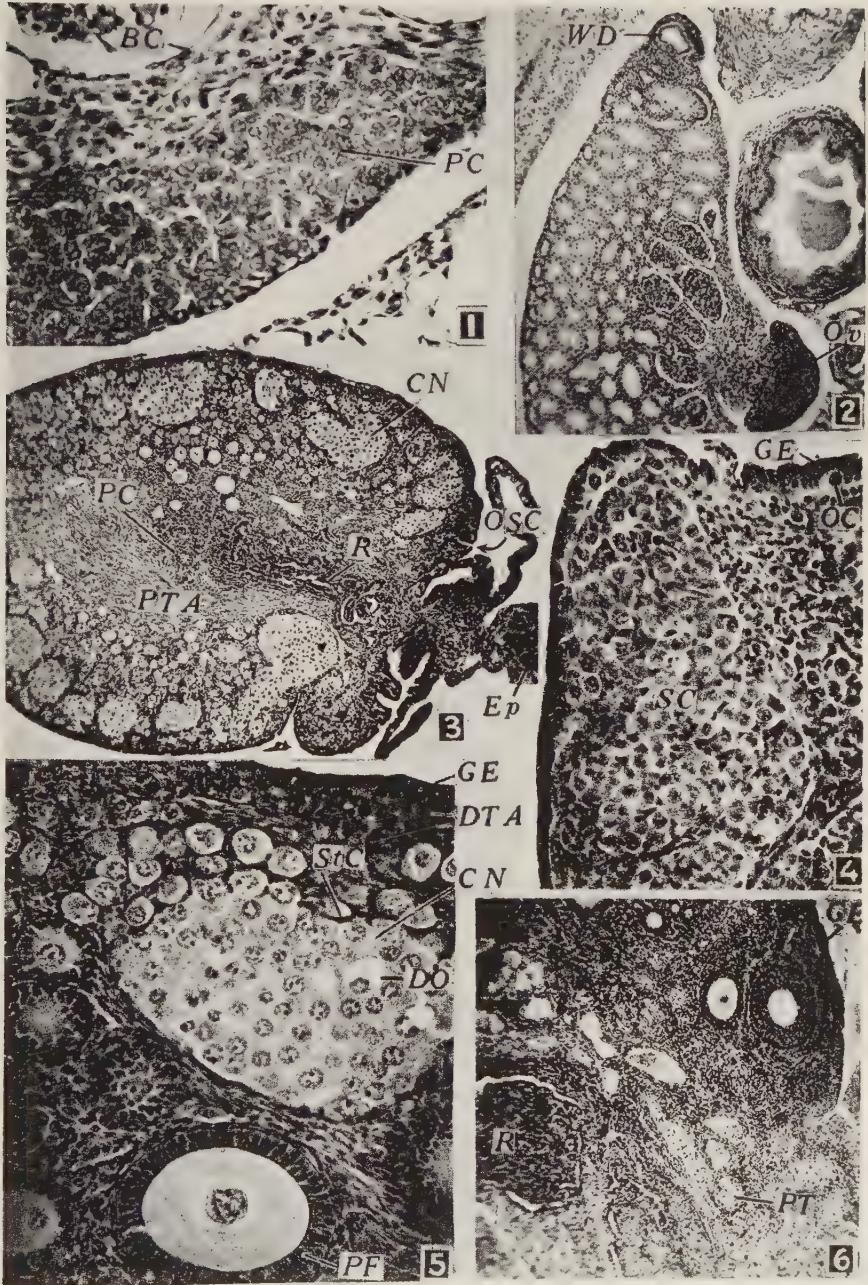


PLATE 2

EXPLANATION OF FIGURES

Ovary of Opossum

7 Normal, age 5 days (1). Showing invaginating secondary cords separated from primary cords by primary tunica albuginea. Note germinal epithelial cells turning in at the invaginations. 400 $\times$.

8 Androgen-treated, age 95 days (118). Rete is shown connecting to primary tubules. Note the single layer of columnar cells of rete, the irregularly arranged cells of primary tubules and the numerous oögonia developing in the primary tubules. 265 $\times$.

9 Normal, age 7 days (4). Rete formation shown by invagination of the peritoneum toward but, in this case, not connected to a short evagination of the epithelium of Bowman's capsule. 265 $\times$.

10 Gonadin-treated, age 17 days (99 B). Short evagination from Bowman's capsule. This connected to rete in a short distance in serial section. Note cells are not typical rete cells (cf. rete in fig. 8). 175 $\times$.

11 Normal, age 240 days (69). Absence of cell nests and follicular antra. Rete is shown. 15 $\times$.

12 Normal, age 100 days (82 B). Polyhedral-shaped cells of oöcyte nests. Note pyknotic cells in nests and cells of nest in late prophase stages of meiosis. 350 $\times$.

13 Normal, age 100 days (62 B). Medullary follicles in primary tubules. The primary tubules connect to rete in next few sections. Ovum in upper center is degenerating. Note how wall of primary tubule is stretched around large ovum, lower center, so that it appears to be a primary follicle. Inner edge of cortical region shown in upper left corner. 225 $\times$.

14 Normal, age 100 days (82 B). Silver preparations showing reticular fibres growing in and surrounding oöcytes. Compare with figure 5. 275 $\times$.

15 Normal, age 24 days (10 A). Rapid appearance of germ cells differentiating from epithelial cells of secondary cords. Note mitotic figure, rete at right and some portions of primary cords containing germ cells. 268 $\times$.

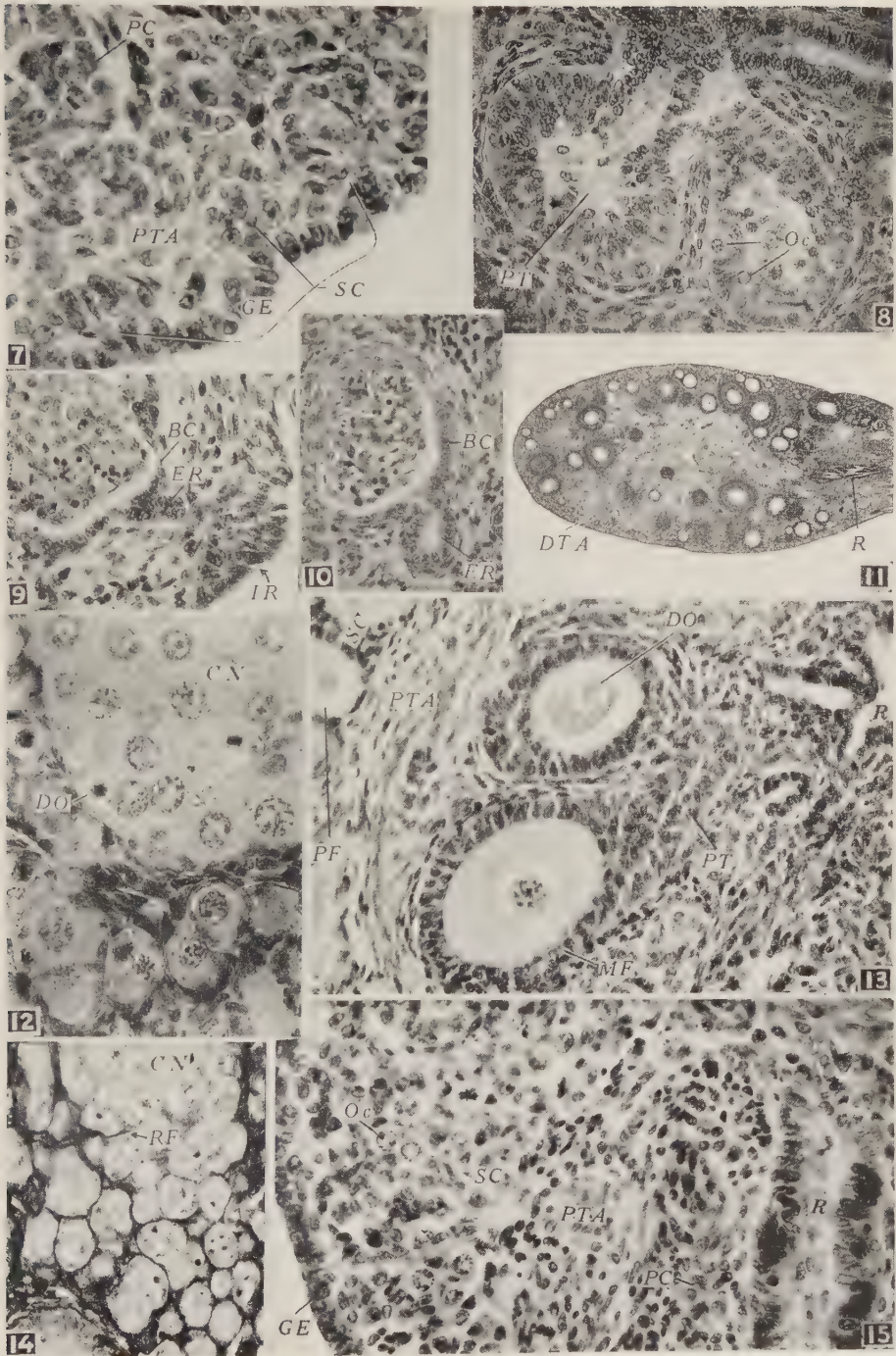


PLATE 3

EXPLANATION OF FIGURES

Ovary of Opossum

16 Normal adult during anestrous season (126 A). Enlarged section of figure 21. Primary tubules connected together. Degenerating ovum in primary tubule upper center. 47 $\times$.

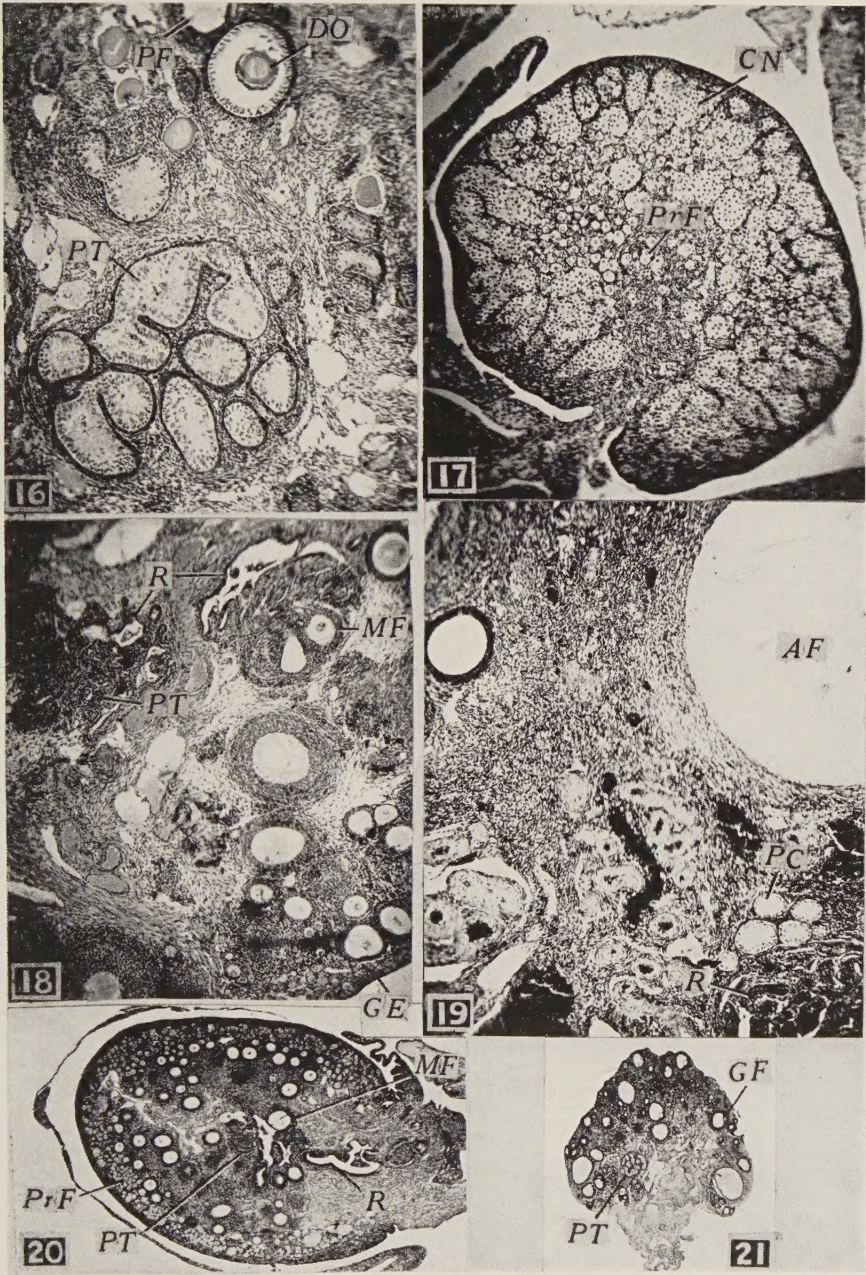
17 Normal, age 44 days (87 B). Showing formation of "cell nests" by connective tissue stromal cells growing into and dividing the secondary cords into "nests." Note primordial follicles. 43 $\times$.

18 Normal, age 150 days (178). Note large follicle, medullary follicles in primary tubules connected to rete in upper center, and absence of "cell nests." 47 $\times$.

19 Androgen-treated adult during anestrous season (125 A), showing large degenerating follicle upper right corner, scarcity of follicles in stromal tissue, primary tubules near cylindrical core of rete tubules lower right. 47 $\times$.

20 Normal, age 100 days (82 B). Many primordial follicles formed from "cell nests." Rete tubules connected to medullary follicles. 20 $\times$.

21 Normal adult during anestrous season (126 A). Note follicles with antrum and primary tubules. (Section of primary tubules enlarged in figure 16.) 4 $\times$.



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